173

A PROSPECTIVE PILOT STUDY OF THYMOGLOBULIN, CYCLOSPORINE (CSA) AND MMF AS GVHD PROPHYLAXIS IN UNRELATED DONOR (URD) HCT USING FLUDARABINE AND MELPHALAN (flu/mel) FOR HIGH-RISK PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Sequential pilot studies at City of Hope using CSA/MMF based prophylaxis in the URD reduced intensity setting have shown high rates of acute GVHD (grade 3-4, 40%; lethal 27%, Rodriguez et al., *Bone Marrow Transplant*, 2004) that are similar to historical controls using CSA or tacrolimus + MTX. Emerging data suggest a lower incidence of GVHD with Thymoglobulin. We are therefore conducting a prospective study adding Thymoglobulin to CSA/MMF for URD HCT using flu/mel; preliminary results are reported. The GVHD prophylaxis consists of CSA 3 mg/kg/d (beginning day −1), MMF 15 mg/kg 3×/d (beginning day 0), and Thymoglobulin 7.5 mg/kg total dose (day −4 to day −1/0). Fifteen patients (pts) have been enrolled, 2 discontinued Thymoglobulin due to infusional toxicities, and 11 are evaluable for acute GVHD. The median age is 50 years (33-63), 10 male, 3 female. Diagnoses include: myelofibrosis (4), B-cell NHL (4), AML (2), Hodgkin (1), myeloma (1), bone marrow failure (1). Stem cell source was all PB; median CD34 cell dose of 7.19×10^6/kg (range 4-19 $\times$ 10 ^{6}). Donor/recipients were HLA allele matched except for 3 pairs with allele mismatch at class I, and one at both class I and DRB1. Manageable infusional toxicities with Thymoglobulin were seen in 11 patients. Durable neutrophil engraftment was seen in all pts with a median time to ANC >500 of 14 days (range 11-22). Day 30 STR analysis in bone marrow MNC was 100% donor in all but 1 pt (94%). With a median follow-up of 6 months (range 1-14 months), 12/13 pts are alive; 1 pt died of diffuse alveolar hemorrhage; 1 pt with diffuse large cell NHL has relapsed, others are in remission. Acute GVHD grade 4, 3 and 2 was seen in 1/11 (9%), 1/11 (9%), and 5/11 (45%) pts, respectively; the only pt with grade 4 GVHD inadvertently received a CD34 cell dose of 19×10^6/kg. All pts responded to treatment. Chronic GVHD has not been observed in any of these pts. EBV reactivation was seen in 3 pts, and all responded to a single infusion of rituximab. CMV reactivation was seen in 2 pts, including one with CMV pneumonia; another had CMV colitis without blood reactivation. No other opportunistic infections have been observed. These preliminary results suggest that Thymoglobulin as given in this protocol is safe but infusional toxicities are common. The incidence of severe acute GVHD appears lower than historical controls; 100-day TRM, chronic GVHD and relapse rates are presently very low; EBV reactivation is manageable and CMV disease has been observed.

174

ALLOREACTIVE MEMORY T CELLS INDUCE CHRONIC GRAFT-VERSUS-HOST DISEASE

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Although it is known that chronic graft-versus-host disease (GVHD) may involve both cellular and humoral immunity, the basic pathophysiology of chronic GVHD remains poorly understood, in part due to a lack of experimental models. We have recently demonstrated that alloreactive memory T cells develop in hosts with acute GVHD and mediate persistent host tissue injury. However, whether alloreactive memory T cell-mediated persistent host tissue injury causes chronic GVHD has not been explored. Using mouse models of allogeneic bone marrow transplantation (allo-BMT), we characterized GVHD pathology in secondary hosts receiving alloreactive memory T cells that developed in primary recipient mice with acute GVHD after allo-BMT. 2×10^5 B6 alloreactive CD4 effector/effector memory (E/EM) T cells isolated from primary GVHD BALB.B mice 35 days after transplantation of donor B6 CD4 and CD8 naive T cells caused clinical GVHD in secondary BALB.B recipients with significantly delayed onset, while administration of BALB.B mice with either 4×10^5 B6 CD4 naive T cells or 4×10^5 CD8 naive T cells failed to induce GVHD. GVHD occurred in secondary BALB.B recipients by day 35, and peaked between days 50 to 70 after transplantation. Consequently, as many as 60% of these secondary BALB.B recipients of alloreactive CD4 E/EM T cells succumbed to GVHD from day 50 to day 90 following adoptive transfer. This was in sharp contrast to the observation that acute GVHD developed in primary BALB.B mice receiving donor B6 CD4 + CD8 naive T cells as early as day 7 after transplantation. Histologic evaluation showed that while both B6 naive T cells (CD4+CD8) and alloreactive CD4 E/EM T cells mediated lichenoid-like cutaneous inflammation at later stage in hosts that survived over 50 days after transplantation, donor naive T cells but not alloreactive CD4 E/EM induced severe acute gastro-intestinal inflammation. Like CD4 E/EM T cells, 4×10^5 alloreactive CD8 E/EM T cells that were isolated from primary GVHD BALB.B mice receiving B6 CD4 and CD8 naive T cells also caused GVHD in secondary BALB.B recipients with delayed onset. In these secondary BALB.B recipients of alloreactive CD8 E/EM T cells, the incidence of severe cutaneous inflammation was significantly higher than primary BALB.B recipients of donor naive T cells. All these results suggest that alloreactive memory T cells responsible for the persistent host tissue injury may cause chronic GVHD.

175

CONDITIONING INTENSITY AND DENDRITIC CELL (DC) ACTIVATION: IMPLICATIONS FOR GVHD CONTROL USING DC DEPLETING ANTIBODIES

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Graft versus host disease (GVHD) is a major limitation to the widespread application of allogeneic haematopoietic stem cell transplantation (HSCT). Dendritic cells (DC) are central to allogeneic transplant interactions. Preparative myeloablative conditioning (MAC) regimens aim to ablate the host immune response and allow haematopoietic reconstitution. Conditioning also facilitates cytokine release and the host DC/donor T cell interactions that initiate GVHD. Reducing the intensity of conditioning (RIC) regimens can maintain immune anti-leukaemic activity and reduce treatment related mortality but the overall incidence of GVHD is unchanged. We hypothesize that this may be due to the increased persistence of host DC. Whilst there is information on the effects of MAC on DC, there is no information on the effects of RIC on DC. We have established murine models of conditioning (MAC = cyclophosphamide [CY] + 800 cGy and RIC = fludarabine + CY + 200 cGy). DC numbers, activation status and subset composition were studied each day of the conditioning regimens. In the absence of donor cells, a significantly higher proportion of plasmacytoid DC (pDC) was documented in mice receiving MAC as opposed to RIC ($P < .001$), but mice that received RIC have significantly higher absolute numbers of host pDC. Subsequent HSCT experiments indicate similar levels of donor DC chimerism between the two conditioning regimens. The question remains as whether the residual host DC are pDC and whether this may explain the delayed GVHD in RIC transplant recipients. Strategic administration of DC depleting antibodies could be an effective means to control GVHD. Intra-peritoneal (ip) injection of N418, a monoclonal antibody to mouse leukocyte integrin CD11c depleted murine DC *in vivo*. Preliminary experiments show elimination of 50% of DC after injection of N418 (500 mg). Subsequent experiments show that 1 mg of N418 is sufficient to significantly delay, but not prevent, GVHD in a full MHC mismatched model of HSCT ($P = .025$). Together, these observations suggest that increasing antibody concentration and prolonged administration may be required to prevent GVHD. The successful application of DC depletion to control GVHD will improve the safety of HSCT for patients with leukaemia.